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Nest cover and faecal glucocorticoid metabolites are linked to hatching success and telomere length in breeding eiders (*Somateria mollissima*)

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ABSTRACT

Habitat-associated crypsis may affect perceived predation vulnerability, selecting for different predator avoidance strategies. Glucocorticoids could mediate the adjustment of escape responses to the extent of crypsis, introducing an overlooked source of variation in glucocorticoid-fitness relationships. However, prolonged exposure to elevated glucocorticoids may be costly leading to accelerated telomere loss and consequently senescence. Here, we examined how nest cover and immunoreactive faecal glucocorticoid metabolite levels (fGCM) are linked to hatching success and telomere length in breeding female eiders (*Somateria mollissima* Linnaeus, 1758). We hypothesized that the degree of nest crypsis, reflecting differences in perceived predation risk, would moderate the relationship between reproductive success and fGCM levels. We also expected that telomere length would be shorter in birds with higher glucocorticoid concentration. Results showed that individuals with high fGCM levels had higher hatching success in nests with low cover, while low fGCM levels were more successful in well-concealed nests. We found that shorter telomeres were associated with high fGCM in nesting sites offering little cover and with low fGCM in well-concealed ones. This study provides the first evidence of habitat-dependent moderation of the relationships between stress physiology, telomere length and hatching success.

Keywords:

Cost of reproduction, eider, glucocorticoids, nesting habitat, *Somateria mollissima*

INTRODUCTION

Glucocorticoid (GC) stress hormones are considered to play a key role in integrating information about environmental challenges and in mediating inter-individual differences in fitness (Ricklefs and Wikelski 2002; Boonstra 2013). So far, however, no consensus has emerged on the direction of the relationship between GCs and fitness in natural populations (Wingfield and Sapolsky 2003; Bonier et al. 2009a, b, Crossin et al. 2012; Jaatinen et al. 2013). This uncertainty is perhaps not surprising given that a negative relationship between GCs and fitness is often assumed *a priori*, based on evidence from biomedical research (Boonstra 2013). Thus, prolonged exposure to high concentrations of GCs is considered to be costly and subject individuals to pathologies (Ricklefs and Wikelski 2002). In contrast to laboratory models, however, wild animals are exposed to a diverse array of stressors, including predation risk. A short-term increase in GC levels is a vital response immediately before, during and after a predatory attack (reviewed by Wingfield et al. 1998). Less known and appreciated is the fact that circulating higher baseline GC concentrations may be adaptive whenever the risk of stress exposure is high ('preparative hypothesis'; Romero 2002), as GCs may prepare the organism to perform better under such circumstances (Sapolsky et al. 2000). Thereby, long-term elevation of GCs may serve to prepare prey for attacks by predators, for instance, by increasing vigilance (Scheuerlein et al. 2001; Cockrem and Silverin 2002; Hawlena and Schmitz 2010). If the benefits of such preparative responses outweigh the costs, an increase in GCs can be adaptive and continue to promote fitness (Boonstra 2013). Therefore, the ambiguity in the relationship between GCs and fitness may partly reflect our incomplete understanding of stress coping strategies in the wild.

The ability of the breeding habitat to provide protection has received little attention as a contextual factor affecting the relationship between GCs and fitness (Crespi et al. 2013; but see D'Alba et al. 2011). The degree of crypsis provided by the nest site is inexorably linked to the optimal predator avoidance

65 tactic. The optimal solution in well-concealed nest sites may be to rely on crypsis and down-regulate
66 escape behaviour (Amat and Masero 2004; Albrecht and Klvaňa 2004), whereas preparing for predatory
67 attacks by maintaining escape performance at a high level may maximize breeding success in less-
68 concealed nests (Merilaita et al. 1999). Such increased vigilance and escape performance has been
69 linked to high baseline GC levels (Sapolsky et al. 2000; Chin et al. 2009; Thaker et al. 2010).
70 Experimental evidence suggests that the nest characteristics themselves may not directly affect the stress
71 hormone levels of breeding birds, but rather that the breeders adopt different strategies of nest-site
72 selection depending on their phenotypic traits (D'Alba et al. 2011). Although a parent selecting a poorly
73 concealed nest site may have high GC levels due to high perceived predation risk, this physiological
74 response may in fact represent an appropriate predatory avoidance strategy enhancing reproductive
75 success (Boonstra 2013). While such a response may be adaptive, increased GCs may still have
76 detrimental effects on the condition and future reproductive potential of individuals in long-lived species
77 (Johnson 2007; Haussmann and Marchetto 2010).

78

79 The ability to cope with external and internal challenges varies widely between individuals (Wilson and
80 Nussey 2010) and this variation may be associated with habitat choice (e.g., D'Alba et al. 2011). Despite
81 this, only a few studies have considered the intrinsic stress tolerance quality of individuals occupying
82 different habitats (e.g., Germain and Arcese 2014). Thus, we still know very little about fitness value
83 associated with a given breeding habitat. Telomeres, nucleoprotein structures located at the ends of
84 chromosomes, hold promise as a composite indicator of physiological stress associated with internal and
85 external challenges (Mizutani et al. 2013; Young et al. 2015; LeVaillant et al. 2015). In general,
86 individuals with longer than average telomeres for their age have longer life expectancy (Heidinger et al.
87 2012; Barrett et al. 2013; Angelier et al. 2013), a higher number of functional cells (Monaghan and
88 Haussmann 2006; Monaghan 2014), and higher stress resistance (Kotrschal et al. 2007). However,

89 chronically elevated GCs can accelerate telomere shortening (von Zglinicki 2002; Epel et al. 2004; Choi
90 et al. 2008; Haussmann and Marchetto 2010). Because the typically high reproductive investment by
91 good-quality individuals may be facilitated by elevated GC levels (Crossin et al. 2012), such investment
92 may incur costs in terms of accelerated telomere attrition (Reichert et al. 2014, Schultner et al. 2014;
93 Sudyka et al. 2014). However, breeding animals interact with their chosen breeding habitat and also
94 telomere dynamics have been linked to habitat choice (Angelier et al. 2013). This adds a previously
95 unappreciated level of complexity to the interrelationships between reproductive success, glucocorticoid
96 stress physiology and telomere dynamics. Increased GC levels may facilitate reproduction and offspring
97 care and can help individuals adjust their antipredatory behavior depending on the habitat-specific risk
98 of predation. Thus, while the immediate benefits of elevated GCs are evident, elevated GC levels have
99 also been shown to carry long-term costs in the form of telomere shortening and subsequently lowered
100 survival (Kotrschalet al. 2007).

101

102 To bring clarity to these issues, we explored potential links between the degree of visual nest
103 concealment and stress physiology, telomere length and breeding success. We hypothesized interactive
104 effects of nest cover and GCs on breeding success: low nest cover is associated with higher perceived
105 predation risk than covered nests and thus the optimal antipredatory response may differ between
106 degrees of nest concealment. We predicted that individuals with higher GC, and thereby presumably
107 enhanced anti-predator responsiveness, would have the greatest reproductive output in poorly-concealed
108 nests facilitating rapid escape, whereas individuals attaining high reproductive success in concealed
109 nests would exhibit lower GC levels and rely on crypsis instead of escape. We also hypothesized that
110 high levels of reproductive performance, either in association with elevated GC levels or independently,
111 may be linked to shorter telomeres (see Bauch et al. 2013).

112

113 As a model system, we used female eider ducks (*S. mollissima*) from a well-studied population in south-
114 western Finland. The eider is an excellent study species. Female eiders rely to a large extent on stored
115 energy resources during incubation (Parker and Holm 1990; Bolduc and Guillemette 2003; but see
116 Hobson et al. 2015; Jaatinen et al. 2016). These limited resources can be mobilized by GCs and
117 consistent individual differences in baseline GC profiles are associated with individual differences in
118 current reproductive success (Jaatinen et al. 2013), and thus potentially also long-term fitness. Also,
119 females show fidelity to nest sites (Öst et al. 2011; Ekroos et al. 2012) and the degree of nest cover is
120 repeatable between years (Öst and Steele 2010; Selmann et al. 2014). Further, incubating females
121 encounter a spatially and temporally varying risk of attack by predators, posing a considerable threat for
122 this ground-nesting bird (Ekroos et al. 2012). The relationship between the acute (handling-induced)
123 stress response and reproductive investment has previously been shown to be modulated by predation
124 risk (Jaatinen et al. 2014). Finally, the number of years of maternal experience, a proxy for age, does not
125 explain the variability in telomere length observed among adult eider females (this study), and thus age
126 is not likely to confound or mask the associations under focus here.

127

128 A previous experimental study on female eiders showed that nest shelter did not affect baseline plasma
129 GC levels (D'Alba et al. 2011). However, this study also suggested that nest habitat was not independent
130 of individual quality and that the relationship between hatching success and CORT was affected by
131 female body condition, i.e., it was state-dependent. This study explores these possibilities further by first
132 assessing the relationships between nest cover, female hatching success and faecal glucocorticoid
133 metabolite (fGCM) level, an accumulative index of stress (Möstl et al. 2005). fGCMs provide a more
134 integrated measure of adrenocortical activity than point serum samples and thus diminish the influence
135 of temporal changes in GC secretion (Whitten et al. 1998). Thereafter, we examined the associations
136 between individual biological state, as quantified by telomere length, nest-site cover and fGCM. In all

analyses we also controlled for other potentially important predictors of hatching success and telomere length, including female breeding experience, body condition and timing of breeding.

MATERIALS AND METHODS

Field methods

(a) Study area and population

The study was conducted at Tvärminne Zoological Station (59°50'N, 23°15'E), in the western Gulf of Finland, in 2009-2011. The 23 study islands were represented by 9 forested island and 14 open rocky islets. Nest cover is highly variable on both island types as females readily nest under trees, bushes such as junipers (which are often abundant also on open islands), rock outcrops or concealed in grassy vegetation. Annually all study islands are searched through with equal thoroughness so that all nesting events are recorded. The number of nests on the islands ranged between 0-94 (mean \pm SD = 15.7 \pm 2.1) during the study period (Jaatinen et al. 2014). Female eiders in the study population nest at low densities and previous evidence suggests that nest-site selection is not affected by female competition or nest-site limitation (Öst et al. 2008; Öst and Steele 2010; Ekroos et al. 2012; Seltnann et al. 2014), which contrasts with the situation described for eiders in other populations that nest in dense colonies (e.g., D'Alba et al. 2011). Thus, in our current sample of individuals, there was no association between nest cover and the onset of breeding (linear mixed model: $b = 0.001$, $SD = 0.002$, $t = 0.303$ $p > 0.05$, N (observations/females) = 472/346) or between nest cover and body condition (linear mixed model: $b = 0.018$, $SD = 0.012$, $t = 1.478$, $p > 0.05$, N (observations/females) = 472/346). Therefore, it is reasonable to assume that individual quality does not create a significant confounding effect on initial nest-site selection, which instead represents the outcome of an active decision-making process when females

come to the area to breed for the first time. In contrast, nest-site choices in subsequent breeding seasons may to some degree be constrained by high fidelity to the particular breeding island (Öst et al. 2011). The low breeding dispersal has been identified as a putative ecological trap for females (Ekroos et al. 2012). Thus, despite an increased propensity to switch nest sites after events of nest depredation (Öst et al. 2011), females still exhibit high breeding philopatry regardless of the increased predation pressure on adults by white-tailed sea eagles (*Haliaeetus albicilla* Linnaeus, 1758), eagle owls (*Bubo bubo* Linnaeus, 1758) and several mammalian predators. On average open islands are subjected to higher predation pressure than islands with a forest cover (Ekroos et al. 2012). In a short term, spatial predation patterns may change due to predator movement between islands and this type of variation is more pronounced than temporal variation in predation pressure (Öst et al. 2011)

(b) Female trapping and measurements

We captured nesting eider females (535 captures of 381 individuals) on their nests by using hand nets. Captured females were weighed with a spring balance to the nearest 10g, measured for structural size (length of the radius-ulna to the nearest 1mm), ringed, and their clutch size was recorded. Clutch size varied between 2-7 eggs (mean \pm SD = 4.69 \pm 1.16). Ducklings are not ringed in this population and hence female age could not be directly determined; therefore the ringing information was used to calculate the number of years since the bird was first trapped, indicating minimum years of maternal experience (Öst et al. 2008; Öst and Steele 2010; Jaatinen and Öst 2011; Jaatinen et al. 2012). This is a reasonably good proxy for female age in the population due to the high breeding philopatry and the fact that more than half of the breeding females in the population are captured annually, with a relatively constant annual trapping effort since 1996 (Jaatinen and Öst 2011).

We obtained female blood samples by extracting approximately 1 ml of peripheral blood from the

brachial vein. Faecal samples were collected in Whirl-Paks (Nasco) directly from the female or by gathering fresh faeces from the nest, and both blood and faecal samples were immediately stored on ice in a cool box and transported to the laboratory within 2–4 *h*. Blood samples were centrifuged in a cold centrifuge (Sigma 3K12, B. Broun, Germany) for 10 min at 1500×*g* to separate blood serum and cells. Blood cells and faecal samples were stored frozen in –20°C until further analyses. Faecal samples collected during 2009–2011 (*N*=514/369) were used for immunoreactive fGCM measurement, whereas blood cells were collected only in 2011 (*N*=197) and subsequently used for telomere measurement.

We used egg floatation to determine the incubation stage at female capture (Kilpi and Lindström 1997). Information on incubation stage was used to calculate a body condition index since eider females refrain completely from feeding during the incubation period and lose up to 40% of their pre-laying body mass (e.g., Parker and Holm 1990). Body condition indices were determined for all trapped females that had been incubating eggs for at least 8 days (egg laying may otherwise not have been completed; Öst et al. 2008). The index was given by the standardized residuals of a regression of log-transformed projected weight at hatching (response variable) on log-transformed radius–ulna length, and indices were derived separately for each year (Öst and Steele 2010). A female’s body mass at hatching was estimated by subtracting an estimate of the expected body mass loss during the remaining incubation time from her measured incubation body mass. Females were weighed once, but as females abstain from feeding during incubation and females were captured at different times in their incubation, we can derive an estimate of average mass loss rate during incubation as the slope of the regression of log-transformed body mass (response variable) on log-transformed incubation time and projected hatching date (Öst et al. 2008). The assumption of continued mass loss after female capture applies to our study population, which makes this index reliable for estimating body condition (Öst and Steele 2010).

209 Hatching success was determined upon subsequent nest visits that were timed to coincide with the
210 expected hatching of the clutch, based on estimated incubation stage at trapping. Successful hatching
211 was determined by observing live ducklings in the nests. If the female had left the nest with her brood
212 prior to our arrival, we observed whether the egg shells remaining in the nest had intact egg membranes,
213 indicating successful hatching (Öst and Steele 2010). Such egg shell remnants can be distinguished from
214 those left after nest depredation. Thus, we were able to precisely determine the fate of the nests for the
215 majority of trapped females ($N=449$ observations (333 females) out of 535 (381 females), 83.9%). Of the
216 nests with known fates in 2009-2011 (Supplementary Table 1), the majority were successful (at least one
217 egg hatched; annual mean $\pm SD = 63.1 \pm 15.1\%$), nearly one-third were depredated ($29.2 \pm 13.3\%$),
218 while only a small fraction were abandoned ($7.7 \pm 6.2\%$). For all nests, we recorded the number of
219 successfully hatched eggs (duckling has hatched and survived to leave the nest) and unhatched eggs (the
220 number of eggs that failed to hatch due to depredation and abandonment, and to a lesser extent
221 inviability). Hatching success of undisturbed eider nests is high at ca 90 %, showing low variability
222 among clutches (Swennen 1989), and thus the small fraction of inviable eggs is unlikely to
223 systematically bias our results. All nests with known fates were included in subsequent analyses (2009-
224 2011; $N=449/333$). To quantify spatial and temporal variation in predation risk, we calculated an annual
225 island-specific predation index. This index was given by dividing the number of depredated nests with
226 the total number of censused nests on a given island in each year (Öst et al. 2011).

227 Nestcover was quantified by taking hemispherical digital photographs with a Olympus C-740 camera
228 equipped with a 42-mm Opteka fisheye lens. All nest photographs were taken right after females had
229 hatched their broods to reduce bias caused by vegetation growth. Hemispherical images were taken by
230 placing an upward facing camera on a stable surface in the nest (Öst and Steele 2010). We used the
231 program Image Tool (v. 3.00; University of Texas Health Science Center, San Antonio) to process nest
232 cover photographs. Firstly, images were converted to grey scale and pixels assigned as black or white so

233 that vegetation and other elements such as rocks providing cover were coded as black pixels whereas
234 areas of open sky were coded as white pixels. Nest cover was then calculated as the proportion of black
235 pixels in the image.

236

237 *Laboratory methods*

238 **(a) Telomere measurement**

239 Relative telomere length normalized for a non-variable copy gene (T/S), was measured in red blood
240 cells (RBC), and corresponds to the average telomere length across chromosomes (Cawthon 2002). The
241 length of telomeres in RBC reflects the telomere length of hematopoietic stem cells (Vaziri et al. 1994)
242 and has been shown to correlate with telomere length in other tissues (Reichert et al. 2013). We obtained
243 a relative telomere length measure (T/S) by using the real-time quantitative PCR (qPCR) method as
244 proposed by Cawthon (2002) and previously validated for use in birds (Criscuolo et al. 2009). Results
245 from the qPCR correlate well with the results obtained by terminal restriction fragment (TRF) analysis,
246 the conventional method of telomere measurement, and qPCR has successfully been used in a growing
247 number of studies (e.g., Criscuolo et al. 2009; Bize et al. 2009; Aviv et al. 2011; Heidinger et al. 2012).

248

249 Genomic DNA for the assay was extracted from 5µl of RBC following the protocol by Aljanabi and
250 Martinez (1997). DNA integrity was determined by agarose gel electrophoresis where 50ng of
251 undigested DNA were resolved in 1.5% agarose gel at 120V for 90min, and DNA purity and
252 concentration was measured spectrophotometrically using a NanoDrop 2000 (ThermoFisher Scientific).
253 Only intact samples, appearing as a tight crown migrating in parallel, and with a A260/A280 ratio >1.7
254 were accepted for further analyses. Because telomere length has not previously been measured in eiders,
255 we first validated the assay. We selected the *gapdh* gene (glyceraldehyde 3-phosphate dehydrogenase) to
256 test whether it could function as the non-variable copy gene in the qPCR assay. Primers which were

originally developed for chickens (genbank accession number: NW_001471525): *gapdh*Fw.: (5'-TCCTGTGACTTCAATGGTGA-3') and *gapdh* Rev.: (5'-AAACAAGCTTGACGAAATGG-3') also successfully amplified the *gapdh* gene fragment in eiders, which resulted in a single DNA band of the expected size (80bp) when visualized on an agarose gel. Negative control reactions, without template DNA, showed no detectable product, suggesting that primer-dimer formation during qPCR was negligible. To confirm that the *gapdh* gene fragment is non-variable in copy number we compared whether the number of copies was stable at the inter-individual level ($N=31$) as well as at the intra-individual (for repeatedly sampled individuals with at least a 10-year gap in sampling, $N=4$). Examination by qPCR showed that the *gapdh* copy number did not differ between individuals and it also did not systematically change with age in the same individuals. These results are also supported by other evidence suggesting that the avian *gapdh* gene is usually a single copy gene found on autosomes and no pseudogenes have been identified (Alström et al. 2011). For amplifying telomeric repeats we used the universal primers developed by Cawton (2002): *tel1b* (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3') and *tel2b* (5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'). After amplification, the products were visualized on agarose gels and we observed a smear which was most intense around 78bp as reported in previous studies (Criscuolo et al. 2009).

274

The qPCR reactions were carried out in BIO-RAD X1000 real time thermal cyclers (BIO-RAD) in 384 well microplates (BIO-RAD). For this purpose, we used iQTM SYBR® Green qPCR mix (BIO-RAD) which includes iTaqTM DNA polymerase, dNTPs, MgCl₂ and fluorescein-SYBR. The reaction mix contained iQTM SYBR® Green qPCR mix, and 400 nM of forward and reverse primers for either telomere and *gapdh* gene fragment amplification. 10 ng of sample DNA was added to each reaction and each sample was measured in triplicate on the same plate. Each plate also included serial doubling

281 dilutions (from 1.25 ng/well to 40 ng/well) of a standard sample DNA plus a no template control, also
282 carried out in triplicate for both telomere and *gapdh* reactions and later used to construct standard
283 curves. The thermal cycling conditions for both amplicons, were as follows: an initial denaturation at
284 95°C for 3 min followed by 45 cycles of 95°C for 15s, 58°C for 18s and 72°C for 30s. The melt curve,
285 used to determine the specificity of the qPCR amplification, was generated by slowly increasing
286 temperature (0.1°C/s) from 65 to 95 °C.

287

288 Our mean qPCR efficiencies, determined from the standard curve, were 102% (± 6.31 SD) and 92%
289 (± 4.9 SD) for telomere and *gapdh* reactions, respectively, and thus fell within the acceptable range (85-
290 115%) for reliable telomere measurements (Criscuolo et al. 2009). Cq values (defined as the cycle
291 number at which the fluorescence reached a fixed threshold value) were standardized for interplate
292 variation using the software GenEx6 (MultiD). Replicates of samples were scanned for outliers ($CV >$
293 5%) and resulted in the exclusion of one sample. Intraplate coefficients of variation for telomeres and
294 *gapdh* Cq values were 1.7% and 0.68%, respectively. Inter-plate CV 's were 2.4% for telomere and 1.15
295 % for *gapdh* Cq values. We calculated the average of Cq values for the replicates and used them to
296 calculate relative telomere length (T/S) for 172 females. It was calculated according using a formula
297 taking qPCR efficiencies into consideration (Pfaffl 2001).

298

299 **(b) Faecal glucocorticoid metabolite analysis**

300 Faecal GCM concentrations provide an integrated measure of faecal hormone profiles accumulated over
301 periods up to several days, since female alarm excreta start to accumulate in the intestinal tract
302 immediately after incubation onset, typically occurring after the second egg is laid (e.g., Andersson and
303 Waldeck 2006). Although females were trapped at different incubation stages, fGCM levels do not
304 systematically change with advancing incubation (Jaatinen et al. 2013). We measured immunoreactive

305 fGCMs by radioimmunoassay (RIA) using a double antibody kit (ImmuChem™ Double Antibody,
306 Corticosterone, 1251 RIA Kit, MP Biomedicals, Orangeburg, NY). The assay was carried successfully
307 for 423 samples from 317 individuals, which were sampled from one to three times during the study
308 period. As a biological validation of the assay, we note that immunoreactive fGCMs in eiders have
309 previously been found to be elevated up to 3 weeks after surgical interventions (Latty, 2008) and
310 repeatable within individuals (Jaatinen et al. 2013). A detailed description of the protocol for the use of
311 this RIA kit for eider fGMC can be found in Jaatinen et al. (2013). Briefly, serial dilutions (1:2 to 1:256)
312 of ten pooled faecal extracts were used for constructing a displacement curve which is parallel to the
313 standard curve. This allowed to determine a faecal dilution (1:8) where binding was close to 60% and
314 which was used for all test samples. Radioactivity of the bound portion was read in gamma counter
315 (Gamma C12, Diagnostic Products, CA). The mean recovery rate of 3H-labeled CORT added to faecal
316 samples pools was $78 \pm 10\%$. The cross-reactivities with other steroids were: desoxycorticosterone
317 (0.34%), testosterone (0.1%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%) and 0.01%
318 for other steroids. The mean sensitivity of the assay for immunoreactive fGCM was 13.4 ng/g (range
319 7.70 to 21.95 ng/g) and the mean ($\pm SD$) fGCM level in samples was 167.81 ± 124.16 ng/g (range 11.2 to
320 757.65 ng/g). Immunoreactive fGCM levels were always above detection limit and our intra-assay *CV*
321 was less than 10% and inter-assay *CV* was 15.28%.

322

323 *Data analysis*

324

325 To elucidate the effects of immunoreactive fGCM level and nest cover on female hatching success, we
326 constructed a generalized linear mixed effect model (GLMM) with a binomial error distribution where
327 hatched eggs of a clutch were considered a success and unhatched eggs a failure. In more detail, we
328 combined the number of hatched and unhatched eggs in each clutch using the “*cbind*” function in R (R

329 Core Team 2013). This procedure combines the number of successes and failures for each clutch and
330 thus produces a clutch-specific hatching proportion, which takes into account the total number of
331 observations (i.e., eggs) used to produce the clutch-specific hatching proportion. This response variable
332 describes the probability by which a given egg hatches (here after, $P(\text{hatch})$) and we tested whether it
333 was explained by nest cover and female fGCM level (Table 1). To reduce statistical bias arising from
334 missing covariates, we included additional variables known to affect female breeding success: female
335 minimum breeding experience, body condition and hatch date. Because predation pressure varies
336 between islands and between years (Öst et al. 2011), we also included island-specific annual predation
337 risk as a covariate in the model. Year was included to account for annual differences in hatching dates
338 and hatching success, which may arise due to factors other than those explicitly considered in the model.
339 To test our hypothesis that nest cover and immunoreactive fGCM may have interacting effects on
340 hatching success, we included the interaction term between fGCM and nest cover in the model. Model
341 selection was done by removing all non-significant variables ($\alpha = 0.05$) using backward stepwise model
342 reduction, where the least significant covariates were removed one at a time until the model contained
343 only significant variables and interactions. The model was fitted using Laplace approximation and
344 female identity was included as a random effect to correct for repeated measurements on the same
345 female in different years ($N = 423$ observations of 317 females; Table 1).

346

347 To study the associations between telomere length, stress physiology and breeding microhabitat, we
348 constructed a linear model (LM) where relative telomere length was explained by immunoreactive
349 fGCM, nest cover and the interaction between these two variables (Table 2). We included minimum
350 maternal experience and body condition as covariates, to account for the potential telomere attrition with
351 advancing age and potential links between telomere length and individual body condition. Telomere
352 length was log transformed to ensure the normality of model residuals. Non-significant ($\alpha = 0.05$) were

removed from the model using backward stepwise model reduction as described above.

To graphically illustrate significant interaction terms, these were analysed *post hoc* using the established method of simple slope analysis (Aiken and West 1991). Predictive trend lines depicted in graphs serve to illustrate significant interaction between two non-discrete predictors. Grouping of females into three categories depending on the concentration of immunoreactive fGCM (low– L, medium – M, high – H) was done after the statistical analyses therefore significance of the interaction is not affected by the grouping of females. In short, regression equations were restructured to reflect the regression of the criterion on one predictor and simple slope regressions were plotted to display the interactions at the mean and 1SD above and below the mean. All statistical analyses were performed in R2.13.0 (R Core Team 2011).

RESULTS

Hatching success

We found that the relationship between immunoreactive fGCM levels and hatching success varied with the degree of nest cover (fGCM \times nest cover interaction: $b = -0.018$; $SE = 0.004$; $Z = -4.187$; $p < 0.001$; N (observations/individuals)=423/317; $R^2_{\text{marg.}} = 0.18$; Table 1, Fig.1). For eider females with low fGCM, hatching success was positively associated with nest site cover. However, the opposite was observed for females with high fGCM levels; high proportional hatching success was associated with low nest cover. Hatching success decreased with advancing hatching date, as it did with increasing island-specific annual predation risk (Table 1). However, minimum maternal experience and body condition were not significantly associated with proportional hatching success, and there was no significant year effect

376 (Table 1).

377

378 *Telomere length*

379 Variation in telomere length was explained by an interaction between female immunoreactive fGCM
380 level and nest cover (fGCM \times nest cover interaction: $b = 0.002$; $SE = 0.001$; $t = 2.014$; $p < 0.05$; $df = 155$;
381 $R^2_{adj.} = 0.03$; Table 2, Fig. 2). Longer telomeres were associated with high nest concealment for females
382 with high fGCM while the opposite trend was observed for low fGCM females. Importantly, we did not
383 detect a significant association between telomere length and female minimum years of maternal
384 experience, and female body condition was likewise not significantly associated with telomere length
385 (Table 2).

386

387 **DISCUSSION**

388 Consistent with the hypothesized role of GCs in adaptively regulating escape responses to the habitat-
389 specific risk of detection by predators, we found that individuals with high immunoreactive fGCM
390 levels had the highest hatching success in nests offering little cover, whereas females with low fGCM
391 profiles had the highest hatching success in well-covered nests (Fig. 1). Thereby, variation in nest-site
392 preferences may facilitate the coexistence of different baseline GC levels in populations subjected to
393 habitat-specific risks of attack by predators (Rivers et al. 2014), cautioning against uncritically assuming
394 a uniformly negative association between baseline GC levels and fitness (Bonier et al. 2009b). While we
395 observed no link between telomere length and a proxy of age in female eiders, we found that shorter
396 telomeres were associated with high fGCM in nest sites with little shelter and with low fGCM in well-
397 concealed ones (Fig. 2). Interestingly, this habitat-associated pattern of telomere dynamics may imply a
398 potential cost of reproduction, since the females with shorter telomeres also had higher reproductive
399 success. This result agrees with that of a recent study on common terns (*Sterna hirundo* Linnaeus,

1758), showing that individuals with short telomeres had higher reproductive performance (Bauch et al. 2013).

The interactive effects of immunoreactive faecal GCs and nest cover on hatching success are consistent with the presence of habitat-specific antipredator strategies. Cross-species comparisons have shown that ecologically similar co-inhabiting species may show contrasting escape tactics when at risk from predation (Lima 1990; Wirsing et al. 2010). Thus, some species always select dense vegetation because of the protection it provides against predators ('cover-dependent escape tactic'), whereas others prefer a clear path of escape to the air ('aerial escape tactic'; Lima 1990). These different antipredatory tactics may also be present within species (Cuadrado et al. 2001; Thaker et al. 2010; Brink et al. 2011). In the case of eiders, evidence suggests that well-concealed nest-sites may be associated with a reduced risk of detection by predators but also potentially higher costs of escape, favouring cover-dependent escape tactics at such nest-sites. First, predation pressure (number of killed females/nesting attempt) is lower and female survival is higher on forested islands than on open ones (Ekroos et al. 2012). Second, it has been experimentally shown that the risk of egg predation decreases with increasing nest cover (Öst et al. 2008), suggesting that concealed nests may attract less attention from visually hunting predators. Third, the presumed benefit of immobility in the presence of predators ('freezing') in densely vegetated habitat is enhanced by the fact that once detected by a predator, dense vegetation may prevent successful escape (Öst and Steele 2010). In contrast, the optimal strategy in poorly concealed nests may be to rely on early escape from predators in anticipation of the higher risk of predator detection (Amat and Masero 2004; Albrecht and Klvaňa 2004).

D'Alba et al. (2011) argued that exposed nest sites are occupied by female eiders of lower phenotypic quality and that the effects of GCs on hatching success appear to vary independent of nest shelter. In line

424 with these conclusions, our own previous work indicated a consistently negative association between
425 immunoreactive fGCMs and hatching success in eiders (Jaatinen et al. 2013). However, our present
426 study showed that a detailed examination of nest-site preferences profoundly changes these conclusions,
427 providing a more nuanced view of the interrelationships between baseline fGCM, reproductive success
428 and breeding habitat. This discrepancy may relate to the orchestrating role of fGCM in simultaneously
429 affecting both reproductive physiology and antipredator behaviours; both of which are intimately linked
430 to reproductive success (Crossin et al. 2016). GCs are associated with the anticipation or awareness of
431 danger when confronted with the threat of predation (e.g., Korte 2001; Cockrem and Silverin 2002).
432 Thus, GCs enhance vigilance behavior (e.g., Romero and Butler 2007) and causally affect flight
433 initiation distance (Thaker et al. 2010). In incubating female eiders, flight initiation distance increases
434 with the magnitude of the acute handling-induced corticosterone (a major GC in birds) response
435 (Seltmann et al. 2012), while handling-induced corticosterone responsiveness decreases with increasing
436 nest cover (Schmidt et al. 2009; Jaatinen et al. 2014). This earlier work also suggests a positive link
437 between enhanced GC responsiveness and reproductive success under high risk of predation (Jaatinen et
438 al. 2014). Our result showing a positive correlation between high fGCM levels and hatching success in
439 poorly concealed nests corroborates this notion, while also suggesting a nest-cover dependent nature of
440 the association. Although the mechanisms underlying a positive association between corticosterone
441 secretion and fitness under high risk of predation remain obscure, it is perhaps pertinent that
442 minimization of incubation time may be particularly beneficial in microhabitats offering limited
443 protection from predators. Thus, experimental evidence suggests that corticosterone shortens incubation
444 time in birds (Schmidt et al. 2009) and female eiders having a long flight initiation distance,
445 characterized by higher stress-induced corticosterone secretion, have a shorter incubation period
446 (Seltmann et al. 2012).

447

448 We were unable to detect any effect of our proxy for female age on telomere length in adult female
449 eiders. This result could be an artefact of selective disappearance, i.e. individuals with shorter telomeres
450 disappearing earlier from the population (van de Pol and Verhulst 2006). However, body condition, a
451 correlate of life expectancy (Ekroos et al. 2012) showing individual repeatability between years
452 (Jaatinen and Öst 2011), was also not significantly associated with telomere length (Table 2). This lack
453 of a relationship between telomere length and body condition adds credence to the possibility that
454 telomere length may not be associated with age *per se* in adult eiders. Likewise, a lack of an association
455 between age and telomere length in adulthood has been found in some other long-lived birds (e.g.,
456 Mizutani et al. 2009; Pauliny et al. 2012; Rattiste et al. 2015), although there are exceptions (e.g., Bize
457 et al. 2009). In this study, we quantified relative rather than absolute telomere length which could
458 potentially mask some between-individual differences in telomere length (see Young et al. 2013).
459 Nonetheless, some studies on long-lived birds where absolute telomere length was quantified also failed
460 to observe telomere shortening with age (e.g., Hall et al. 2004). Potentially, this lack of correlation may
461 be attributed to lifelong persistence of active telomerase (Haussmann et al. 2007), a possibility
462 warranting further investigation.

463

464 How can we reconcile the finding that individuals with short telomeres had higher breeding performance
465 (Fig. 2) with the widely-held notion that individuals with longer telomeres, after controlling for any age
466 effects, are of higher phenotypic quality (Pauliny et al. 2006; Le Vaillant et al. 2015)? However, as
467 argued by Bauch et al. (2013), increased investment in reproduction may induce telomere loss, and this
468 effect may become particularly pronounced if some individuals consistently perform better than others
469 throughout their lives. Viewed in this light, individual variation in telomere length may implicate long-
470 term cumulative reproductive costs, rather than merely reflecting the current reproductive burden
471 (Bauch et al. 2013). This argument may also be valid in the case of eiders. For example, female identity

472 explains more than half of the variation in nest fate (i.e., at least one egg hatched vs. all eggs unhatched)
473 (Öst and Steele 2010). Nevertheless, some open questions remain, the solution of which will require
474 further, preferably experimental evaluation. One particular challenge relates to the observation that
475 females with low fGCM levels in covered nests had shorter telomeres, given the alleged role of
476 glucocorticoids in accelerating telomere loss (Haussmann and Marchetto2010). Although this effect may
477 seem small (Fig. 2), it deserves further longitudinal study, because our current, cross-sectional analysis
478 inevitably only provides a snapshot of telomere length, and thus it cannot unveil the underlying
479 complexity of the telomere shortening and restoration process (Monaghan and Haussmann 2006).
480
481 Because behavioural reactivity and physiological stress coping mechanisms are tightly linked (Koolhaas
482 et al. 1999), the same forces are likely to maintain variation in both set of traits in the population.
483 Theory predicts that individuals with higher GCs should perform better under unpredictable
484 environmental conditions, whereas low GC levels are favoured under stable conditions (Cockrem 2005).
485 These fundamental context-dependent differences in optima could serve to maintain phenotypic
486 variability in the population under temporally or spatially fluctuating selection pressures. In line with
487 this general expectation, our study demonstrates that short-term reproductive output tends to become
488 equalized for individuals with different stress profiles if individually repeatable habitat choices are taken
489 into account. Nest cover is an important habitat feature especially for ground-nesting birds, as it can
490 influence adult and egg predation risk (Martin 1993), offer variable thermal conditions (e.g., Kilpi and
491 Lindström 1997) and thereby influence habitat predictability. Our current results showed that females
492 with high fGCM have higher reproductive success but shorter telomeres in open compared to concealed
493 nest sites. Since concealed nest sites are less exposed to weather extremes (Kilpi and Lindström 1997;
494 Fast et al. 2007) and predator attacks (Ekroos et al. 2012) and thereby likely to offer a more stable
495 environment, our findings agree with the hypothesis that variation in environmental predictability can

496 promote the co-existence of different behavioural and physiological phenotypes within the same
497 population (Cockrem 2005).

498

499 In summary, we have demonstrated that the relationships between breeding microhabitat, telomere
500 length and reproductive success may differ depending on individual stress coping strategies in a wild
501 population, subject to temporally and spatially varying predation pressure. Our results are consistent
502 with adaptive adjustment of GC levels to match local environmental conditions, thereby tending to
503 equalize fitness across nests of different concealment. Accordingly, our results may help to explain the
504 considerable variation in nest concealment at the intraspecific level (Öst and Steele 2010). Here we have
505 argued that this adjustment may be driven by threat-sensitive predation avoidance, where different
506 behavioural tactics are favoured in contrasting nest microhabitats. However, since our study is
507 necessarily correlational, causality remains to be demonstrated (but see D’Alba et al. 2011). Equally
508 unclear at this point is whether female eiders with high reproductive success, incurring an apparent cost
509 in terms of telomere shortening, also have shorter lifespan, i.e., whether they actually pay a cost of
510 reproduction. In fact, circumstantial evidence suggests a positive relationship between fecundity and
511 survival in this species (Yoccoz et al. 2002). To address these open questions, we encourage future
512 longitudinal studies investigating within-individual relationships between stress physiology, fitness and
513 telomere dynamics, preferably involving experimental manipulations of predation risk.

514

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737 **Table1**

738 Model selection and GLMM (binomial error distribution, log link function and female identity as a
739 random factor) testing the effects of a set of independent variables on proportional hatching success
740 (P(hatch)).

741

Independent variable	Parameter estimate (<i>b</i>)	<i>SE</i>	<i>Z</i> value	<i>p</i>	<i>N</i>
Minimum maternal experience (years)	0.036	0.065	0.555	0.58	421/316
Body condition	-0.118	0.174	-0.675	0.50	408/309
Year	0.359	0.215	1.669	0.09	423/317
Hatching date	-0.226	0.025	-8.981	< 0.001	423/317
Island-specific predation	-4.812	1.433	-3.358	< 0.001	423/317
Nestcover	2.485	0.933	2.662	< 0.001	423/317
fGCM (ng/g)	0.007	0.003	2.434	0.01	423/317
fGCM × nestcover	-0.018	0.004	-4.187	< 0.001	423/317

742 The final model (in **bold**) was selected by removing all non-significant variables ($\alpha = 0.05$). Variables
743 included in the initial model included the *a priori* defined two-way interaction between fGCM and nest
744 cover. Abbreviations: fGCM –faecal glucocorticoid metabolites; df-degrees of freedom; N:
745 observations/unique individuals; SE-standard error. Sample sizes differ because data were not available
746 for all independent variables.

747

749 **Table 2**

750 Model selection and linear model (LM) testing the effects of the set of independent variables on
751 telomere length.

Independent variable	Parameter estimate (<i>b</i>)	<i>SE</i>	<i>t</i> value	<i>df</i>	<i>p</i>
Minimum maternal experience (years)	0.008	0.011	0.697	154	0.49
Body condition	0.017	0.043	0.404	136	0.69
fGCM (ng/g)	-0.001	0.001	-1.274	155	0.20
Nest cover	-0.365	0.310	-1.179	155	0.24
fGCM × nestcover	0.002	0.001	2.014	155	<0.05

The final model (in **bold**) was selected by removing all non-significant variables ($\alpha = 0.05$). Variables included in the initial model included the *a priori* defined two-way interaction between fGCM and nest cover. Abbreviations: fGCM: faecal glucocorticoid metabolites; *df*: degrees of freedom; *SE*: standard error.

760 Figure legends

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762 Fig. 1. Proportional hatching success is affected by an interaction between proportional nest
763 cover and immunoreactive faecal glucocorticoid metabolite (fGCM, ng/g) level, so that the hatching
764 success of females with low fGCM (mean – 1SD, solid line, L, black dots) positively correlates with
765 increasing proportional nest cover, whereas females with high fGCM (mean + 1SD, dotted line, H, open
766 circles) tend to have lower hatching success in concealed nests. Females with intermediate fGCM
767 concentrations (mean, dashed line, M, grey dots) exhibit an intermediate response.

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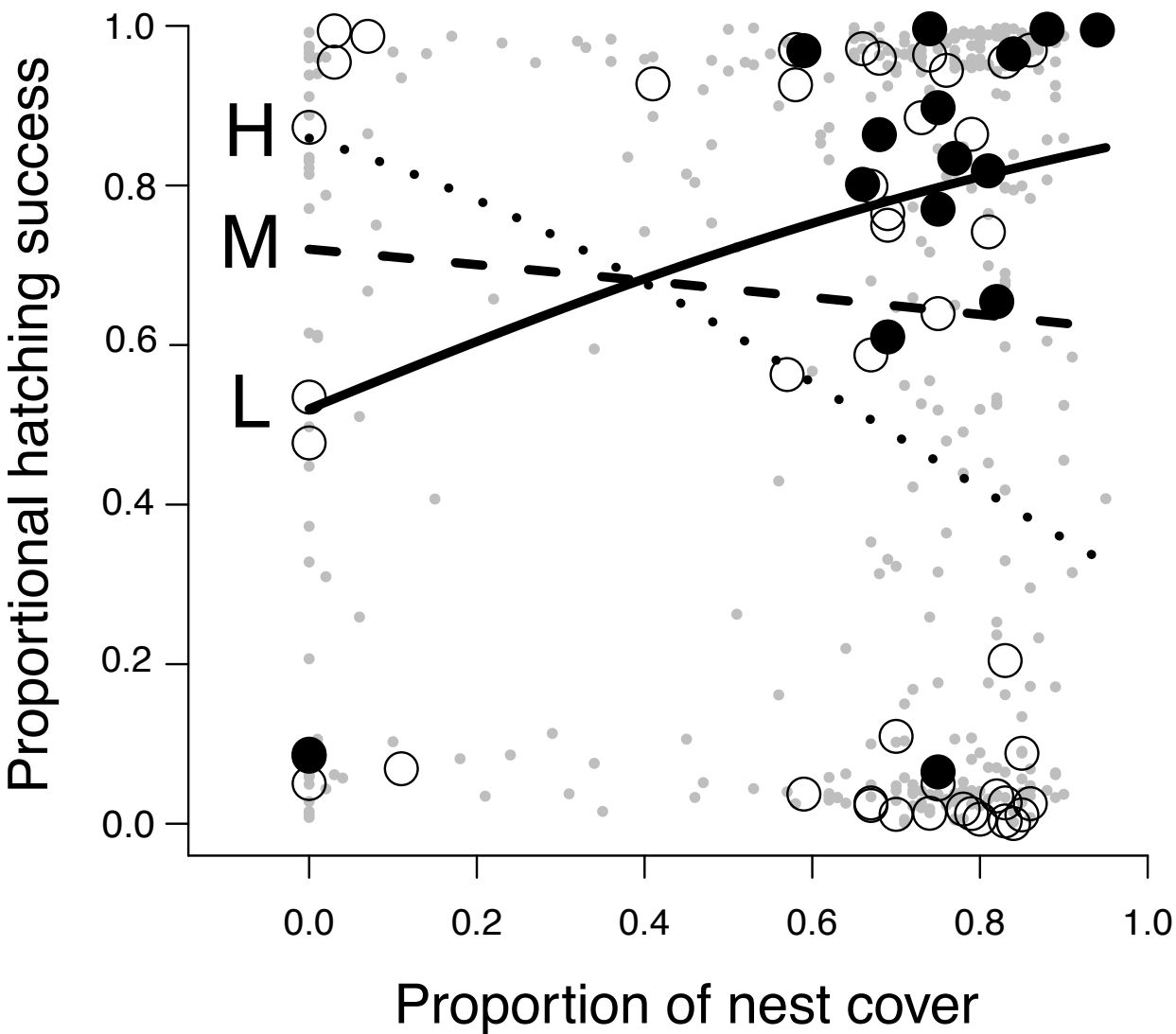
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771 Fig. 2. Female telomere length is connected to nest cover, but this relationship is modulated by the
772 immunoreactive faecal glucocorticoid metabolite (fGCM, ng/g) level. Telomere length is positively
773 associated with proportional nest cover for females with high fGCMs (mean+1SD, dotted line, H, open
774 circles), whereas for nesting females with low fGCM (mean-1SD, solid line, L, black dots) this
775 association is negative. Females exhibiting intermediate fGCM levels (mean dashed line, M, grey dots)
776 show an intermediate response.

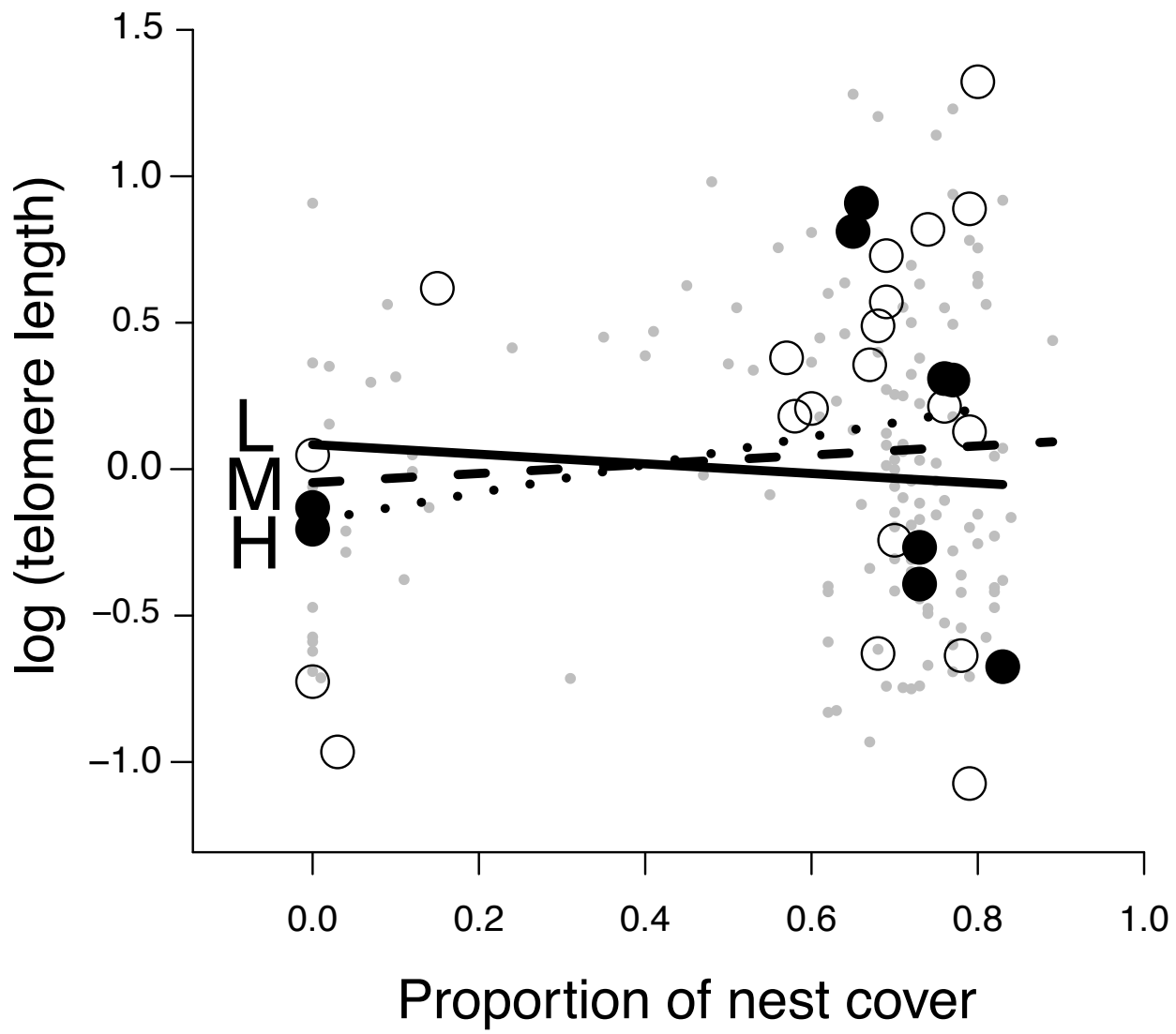
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784 **Supplementary Table1**

785 Nest fate during 2009-2011. Nest fate was categorized as either depredated, abandoned or successful (at
786 least one successfully hatched offspring) (see Methods).

Year	Depredatednest s	Abandoned nests	Successful nests	Total number of known-fate nests	Total number of nests
2009	22 (14.76%)	7 (4.70%)	120 (80.54%)	149	165
2010	43 (31.85%)	20 (14.81%)	72 (53.33%)	135	173
2011	67 (40.60%)	6 (3.64%)	92 (55.75%)	165	197

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